




First records from Cape Verde and range extension of *Coniophora eremophila* Lindsey & Gilb. (Basidiomycota, Boletales): a morphological and molecular identification

MARGARITA DUEÑAS^{1*}, MARÍA P. MARTÍN¹, M. TERESA TELLERIA¹

¹ Department of Mycology, Real Jardín Botánico – CSIC, Madrid, Spain • MD: mduenas@rjb.csic.es  <https://orcid.org/0000-0003-0621-8003> • MPM: maripaz@rjb.csic.es  <https://orcid.org/0000-0002-1235-4418> • MTT: telleria@rjb.csic.es  <https://orcid.org/0000-0002-9876-6914>

* Corresponding author

Abstract. In the framework of a research project on corticioid fungi (Basidiomycota) from Cape Verde, we collected several specimens provisionally assigned to *Coniophora* sp. On the basis of morphological and molecular analyses, we identified these specimens as *C. eremophila* Lindsey & Gilb. These records extend the geographical distribution of this species by approximately 8,800 km in a straight line west to east, from its previously known North American locality and 8,700 km southwest to northeast from the other known locality in Chile. The presence of the genus *Coniophora* is reported for the first time in the Cape Verde Archipelago.

Keywords. Corticioid fungi, Fogo Island, geographic distribution, Macaronesia

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Introduction

The Cape Verde archipelago is situated in the North Atlantic Ocean between 14°45'N, 022°40'W and 17°10'N, 025°20'W and together with the Azores, Madeira, and the Canary Islands form the Macaronesian region. Cape Verde is composed of 10 volcanic islands with varying sizes, topography, and altitudes. The archipelago is spread over more than 58,000 km², has a surface of 4,020 km², and is located 570 km off the coast of Senegal, West Africa. The islands are usually classified in three groups: Northern Islands (Santo Antão, São Vicente, Santa Luzia, and São Nicolau), Southern Islands (Santiago, Fogo, and Brava), both characterized by hilly landscapes, and the Eastern group (Sal, Boavista, and Maio) with flat landscapes. The climate is tropical dry to semi-arid, with annual precipitations of 80–300 mm in arid coastal zones to 1,200–1,600 mm in the highlands; the mean annual temperatures range

from 23–27 °C at sea level to 18–20 °C at high altitude (Duarte and Romeiras 2009).

There is little information about the mycobiota from Cape Verde. A preliminary list of non-lichenized fungi (Bañares Baudet 2005) recorded 58 species, and only seventeen basidiomycetes were included. Recently, three new species of gasteroid fungi (Crous et al. 2015, 2016; Martín et al. 2015) and two of corticioid fungi (Telleria et al. 2017) have been described from Santiago, Santo Antão, São Vicente, and Fogo Islands.

Coniophora DC. ex Merat is a widespread genus of corticioid fungi (Basidiomycota, Boletales, Coniophorinae; Binder and Hibbett 2006). It is characterized by the following morphology: basidioma resupinate, effused, adnate, and membranaceous; hymenophore smooth to tuberculate and margin with hyphal strands; hyphal system mono-, di- or trimitic, most generative hyphae clampless, however with scattered double or verticillate clamps especially in the wider marginal

hyphae; basidia clavate, cylindrical to utriform, two or four sterigmata, without basal clamps; spores broadly ellipsoid, ellipsoid to ovoid, or subfusiform, apiculate; apical germ pore present, thick-walled, smooth, yellowish to brownish and more or less dextrinoid (Ginns 1982; Hallenberg 1985).

Carlier et al. (2004) included 18 species and two varieties in the genus. Since then, one new species has been described (Boidin and Gilles 2004) and a new combination proposed (Parmasto 2005). The geographic distribution of *Coniophora* species is imperfectly known (Ginns 1982), and from the Macaronesian region, the genus has been reported from the Azores (Telleria et al. 2009), Madeira (Telleria et al. 2008), and the Canary Islands (Beltrán-Tejera et al. 2013, 2015); therefore, the new records reported here are the first time this genus has been found in the Cape Verde Archipelago.

Coniophora eremophila was described by Lindsey & Gilbertson (1975) from the Sonoran Desert of Arizona, USA, where it was associated with brown rot on dead mesquite (*Prosopis juliflora* (Sw.) DC.) and other fallen debris of *Olneya tesota* A.Gray, *Sambucus mexicana* C.Presl ex DC., *Chilopsis linearis* (Cav.) Sweet, and *Juglans major* (Torr.) A.Heller. Currently this species is only known from the Sonoran Desert in North America (Lindsey and Gilbertson 1975; Gilbertson et al. 1976) and Punta Arenas, Chile, South America (Ginns 1982).

Methods

The specimens studied in this paper were collected in the Cape Verde archipelago within the framework of a project on the biogeography of corticoid fungi (CGL2009-07231). The field exploration took place on March 2010 on four islands: Santiago, Fogo, São Vicente, and Santo Antão (Figs. 1, 2). The specimens were collected by M. Dueñas (MD) and M.T. Telleria (Tell.) and deposited in the mycological collection (MA-Fungi) of the Real Jardín Botánico Herbarium, Madrid, Spain. This study included type specimens of *Coniophora eremophila* from Arizona (RLG 10925, holotype, BPI) and *C. bimacrospora* Decock, Bitew & G. Castillo from Ethiopia (MUCL 45009, holotype), and additional materials of the ARIZ and MA-Fungi herbaria (Table 1).

The morphological analysis of the species was based on dried specimens; colors of basidioma are given according to ISCC-NBS Centroid Color Charts (Kelly and Judd 1976). Measurement was made from microscopic sections mounted in 3% KOH, Congo red, and Melzer's solutions, and examined at magnifications up to 1,250× using a differential interference contrast Olympus BX51 microscope. The length and width for 30 spores and 10 basidia were measured from each sample, and length/width ratios (Q) were calculated. Drawings were made with a Leica 2500 microscope with the help of a drawing tube.

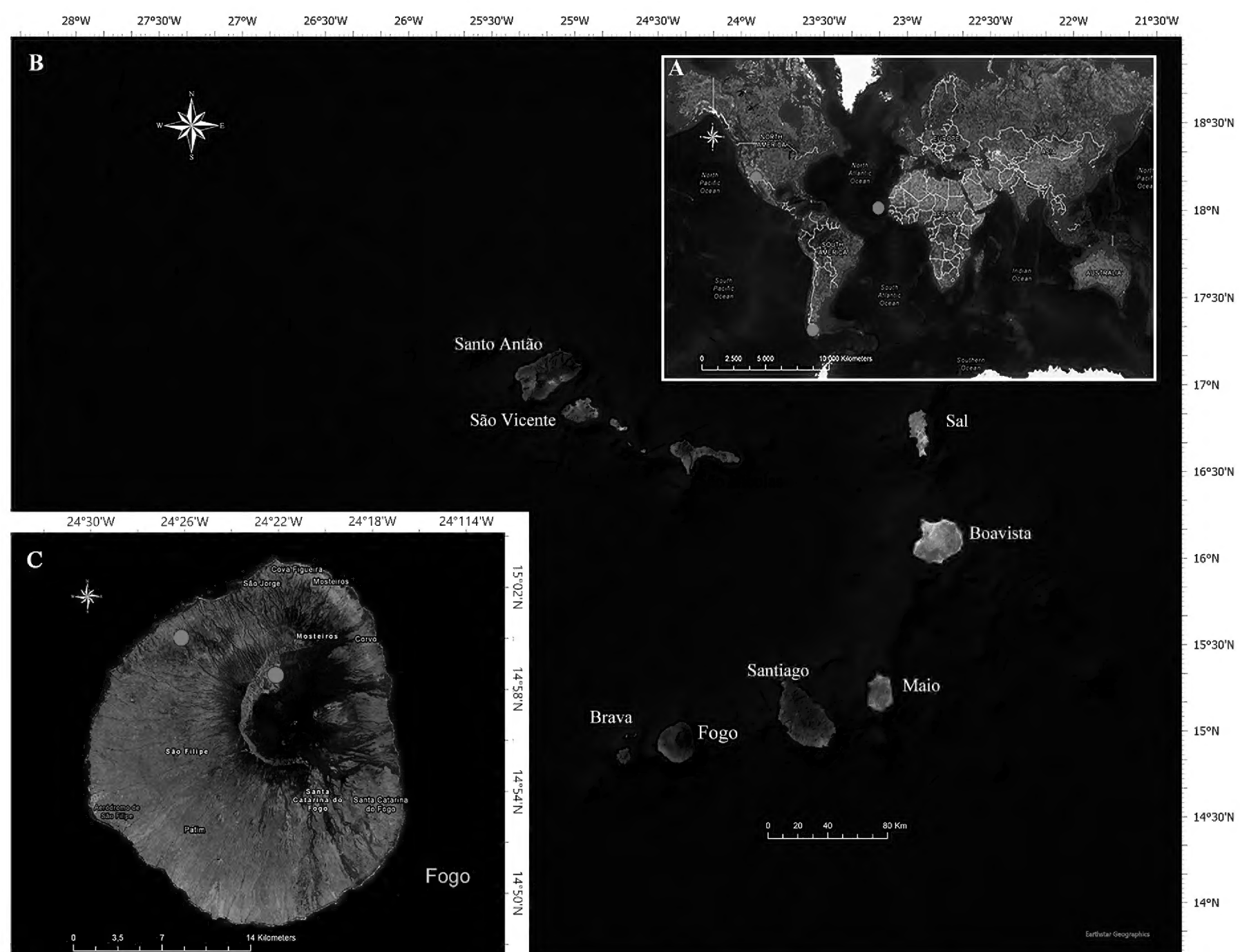


Figure 1. Distribution of *Coniophora eremophila*. **A.** Yellow circles = previous records, red circles = new records from Cape Verde. **B.** Cape Verde Archipelago map. **C.** New records of *C. eremophila* on Fogo Island.



Figure 2. Caldeira do Fogo, where *Coniophora eremophila* 19021 Tell. and 19015 Tell. were located (photographs by M.T. Telleria).

DNA extractions, ITS nrDNA amplifications, purifications, and sequencing protocols were performed as indicated by Telleria et al. (2017). Sequences obtained in this study were submitted to GenBank under the accession numbers indicated in Table 1. The newly generated ITS sequences were aligned in Se-Al v. 2.0a11 Carbon (Rambaut 2002) with homologous sequences retrieved from EMB/GenBank/DDBJ databases (Cochrane et al. 2011, 2016) included in Table S1. The maximum-parsimony (MP) and maximum-likelihood (ML) analyses were performed as by Telleria et al. (2017) using PAUP* v. 4.0b10 for Macintosh (Swofford 2003); GTR+I+G was the model selected in PAUP to ML analysis. Two

sequences of *Serpula himanthioides* (Fr.) P. Karst. and *Serpula lacrymans* (Wulfen) J. Schröt. were included as the outgroup.

Results

Coniophora eremophila Lindsey & Gilb. 1975

Mycotaxon 2: 86 (1975)

Figures 3, 4

New records. CAPE VERDE – **Fogo** • São Filipe, Caldeira do Fogo, Bangaeira; 14°58'40"N, 024°22'03"W; 1666 m alt.; 22.IX.2010; M.T. Telleria leg.; on dead fallen plant debris; 19021Tell., MA-Fungi 86371

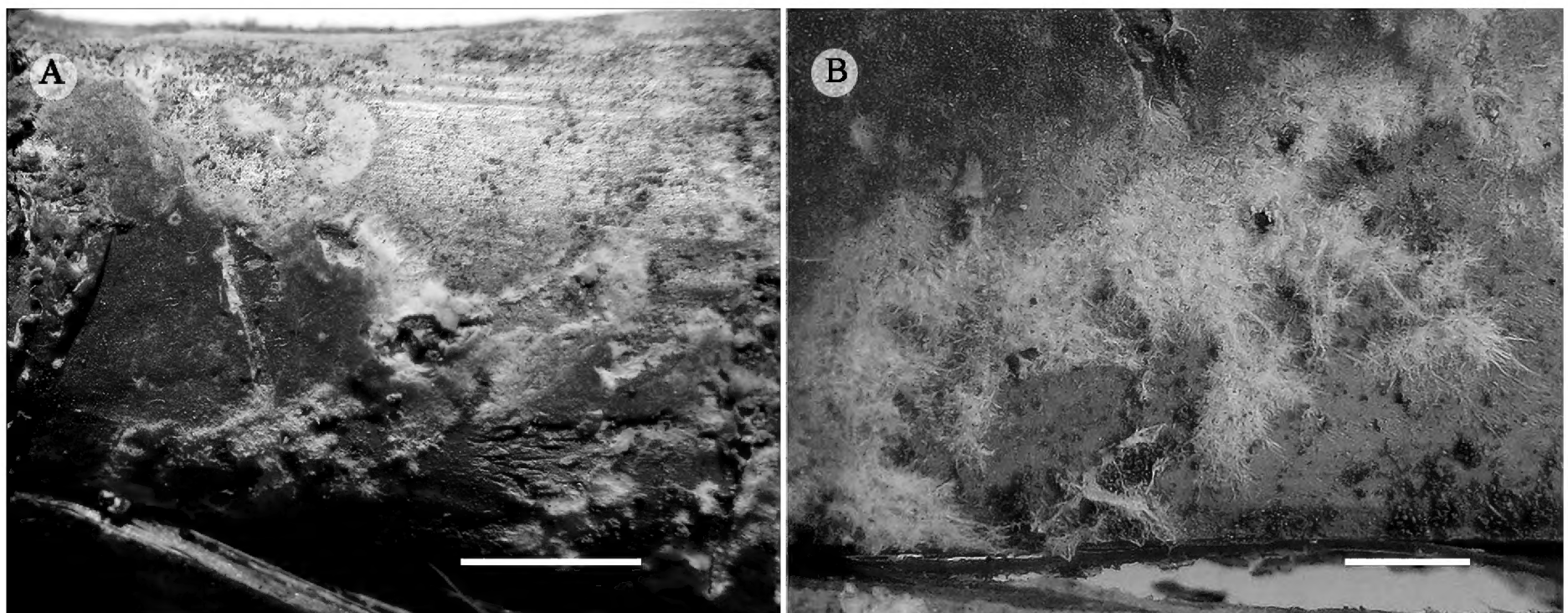


Figure 3. *Coniophora eremophila*, 13284MD, MA-Fungi 86372. **A.** Basidioma. **B.** Basidioma with margin detail. Scale bars: A = 5 mm, B = 1 mm. (Photographs by M. Dueñas)

Table 1. Species and specimens used to reconstruct the phylogenetic tree, with their herbarium or isolate numbers, country, substrate and GenBank accession numbers. * = DNA isolated from mycelium; † = from USDA forest products; ‡ = from xylem; no asterisk: isolated from basidioma.

Species/specimen/isolate	Country	Substrate	GenBank no.	Reference
<i>Coniophora arida</i> (Fr.) P. Karst.				
P294 (Fischer 373)	Germany	No data	AJ344113*	Schmidt et al. 2002
P 232 (MUCL 30844)	United Kingdom	<i>Pinus</i> sp.	AJ345007*	Schmidt et al. 2002
MA-Fungi 39709, 8373MD	Spain	<i>Juniperus thurifera</i>	AJ419194	Martín and Ralid 2002
MA-Fungi 47708, 14057Tell.	Spain	<i>Castanea sativa</i>	AJ419196	Martín and Ralid 2002
MUCL 30844	United Kingdom	<i>Pinus</i> sp.	AM747497*	Kauserud et al. 2007
472	New Zealand	<i>Metrosideros excelsa</i>	AM747498*	Kauserud et al. 2007
FP 105382	USA	Red or white pine	AM747502*	Kauserud et al. 2007
MUCL 40342	Zimbabwe	<i>Pinus</i> sp.	AM747504*	Kauserud et al. 2007
DAOM 52839	Canada	Conifer	AM747511*	Kauserud et al. 2007
FP 104424	USA	<i>Pinus</i> sp.	AM747515*	Kauserud et al. 2007
FP 103793	USA	Hardwood branch	AM747516*	Kauserud et al. 2007
MUCL 31038	Canada	<i>Acer</i> sp.	AM747517*	Kauserud et al. 2007
olrim507	Sweden	<i>Picea abies</i>	AY805608*	Menkis et al. 2004
MUCL 30844 (under <i>C. arida</i> var. <i>suffocata</i>)	United Kingdom	<i>Pinus</i> sp.	GU187511	Binder et al. 2010
CFMR FP-104367	USA	Hardwood	GU187510†	Binder et al. 2010
CFMR HHB-17606	USA	<i>Picea glauca</i>	GU187518†	Binder et al. 2010
MA-Fungi 37397, 10440Tell.	Spain	Unidentified wood	HF921461	This study
MA-Fungi 60590, 10285MD	France	<i>Pinus nigra</i>	HF921462	This study
MA-Fungi 63353, 10320IS (under <i>C. arida</i> var. <i>suffocata</i>),	France	<i>Abies</i> sp.	HF921463	This study
MA-Fungi 11400, 7198Tell.	Norway	<i>Picea</i> sp.	HF921464	This study
VL389	Lithuania	<i>Pinus mugo</i> , xylem	JF440569‡	Lygis et al. (unpublished)
<i>C. bimacrospora</i> Decock, Bitew & G. Castillo				
MUCL 45009, DeCock ET03/072, holotype	Ethiopia	Wood	MK677453	This study
<i>C. eremophila</i> Lindsey & Gilb.				
US 0290491	USA	Unidentified wood	HF921465	This study
MA-Fungi 86371, 19021Tell.	Cape Verde, Fogo	Dead fallen plant debris	HG326617	This study
MA-Fungi 86372, 13284MD	Cape Verde, Fogo	<i>Saccharum officinarum</i>	HG326618	This study
AN 000728, 10875 RL Gibertson	USA	<i>Oleña tesota</i>	HK677454	This study
AN 000731, 10812 RL Gilbertson	USA	<i>Oleña tesota</i>	HK677455	This study
AN 000734, 10894 RL Gilbertson	USA	<i>Juglans major</i>	HK677456	This study
AN 000735, 10936 RL Gilbertson	USA	<i>Sambucus caerulea</i>	HK677457	This study
<i>C. fusispora</i> (Cooke & Ellis) Cooke				
MA-Fungi 7302, 1474Tell.	Spain	<i>Pinus pinaster</i>	HF921466	This study
MA-Fungi 57734, 15702Tell.	France	Unidentified wood	HF921467	This study
<i>C. hanoiensis</i> Pat.				
L. Ryvarden 24995	Zimbabwe	<i>Pinus</i> sp.	HF921468	This study
<i>C. marmorata</i> Desm.				
Isolate P158	Germany	Mining timber	AJ518879*	Schmidt et al. 2002
P 307	United Kingdom	Underside spruce	AJ518880*	Schmidt et al. 2002
FPRL 410, IMI 387582	United Kingdom	Spruce floorboard	AM946632*	Schmidt and Moreth 2008
Isolate PBe10Cm	France	Infected building	GU066836*	Maurice et al. 2011
MUCL 31667	Belgium	House, plaster work	GU187515	Binder et al. 2010
CFMR Braz-6 (under <i>Coniophora</i> sp.)	Brazil	<i>Araucaria angustifolia</i>	GU187517†	Binder et al. 2010
<i>C. mollis</i> Ginns				
PREM 36877, holotype	South Africa	Wood on under-ground limber	HF921469	This study
<i>C. olivacea</i> (Fr.) P. Karst.				
P 297 (Fischer 713)	Germany	No data	AJ345009*	Schmidt et al. 2002
P151	No data	No data	AJ344112*	Schmidt et al. 2002
MUCL 20566	Germany	No data	AM747518*	Kauserud et al. 2007
P154	Germany	No data	AM747519*	Kauserud et al. 2007
82-34/3	Norway	<i>Picea abies</i>	AM747520*	Kauserud et al. 2007

Species/specimen/isolate	Country	Substrate	GenBank no.	Reference
FP 100334	USA	<i>Picea engelmannii</i>	AM747523*	Kauserud et al. 2007
FPL 1	United Kingdom	<i>Thuja plicata</i>	AM747530*	Kauserud et al. 2007
P 161	Canada	No data	AM747531*	Kauserud et al. 2007
DAOM 189127	Australia	<i>Eucalyptus marginata</i>	AM747532*	Kauserud et al. 2007
L-9712	USA	<i>Pinus</i> sp.	AM747537*	Kauserud et al. 2007
CFMR FP-104386	USA	<i>Pinus</i> log down	GU187516‡	Binder et al. 2010
CFMR FP-105969 (under <i>C. prasinoides</i>)	USA	Old flooring cf. <i>Pinus palustris</i>	GU187519	Binder et al. 2010
MA-Fungi 26309, 10369Tell.	Portugal	<i>Pinus pinea</i>	HF921471	This study
MA-Fungi 7829, 1453Tell.	Spain	<i>Pinus pinaster</i>	HF921470	This study
MA-Fungi 26138, 11374Tell.	Portugal	<i>Pinus pinaster</i>	HF921472	This study
MA-Fungi 21282-2, 4176MD	Spain	<i>Cupressus</i> sp.	HF921473	This study
MA-Fungi 70495, 10651IS	France	<i>Fagus sylvatica</i>	HF921474	This study
MA-Fungi 26309, 10369Tell.	Portugal	<i>Pinus pinea</i>	HF921471	This study
C. opuntiae Telleria				
AH31855	Spain	<i>Opuntia ficus-indica</i>	FJ790314	Blanco et al. 2009
MA-Fungi 6901, 4460 Tell., holotype	Spain	<i>Opuntia</i> sp.	HF921475	This study
C. prasinoides (Bourdote & Galzin) Bourdote & Galzin				
MA-Fungi 19417, HH McKay	USA	Pine flooring stored	AJ419197	Martín and Raidl, 2002
C. puteana (Schumacher.) P. Karst.				
P 159	Sweden	Timber 1957	AJ249503*	Moreth and Schmidt 2000
P 153 (FPRL 11e)	United Kingdom	No data	AJ344110*	Schmidt et al. 2002
MA-Fungi 10672	Spain	<i>Pinus</i> sp.	AJ419198	Martín and Raidl 2002
LISU 1782237 (IM79851)	Spain	Wood	AJ419199	Martín and Raidl 2002
FP 100258	USA	<i>Picea engelmannii</i>	AM293045*	Kauserud et al. 2007
DAOM 17535	Canada	<i>Picea glauca</i>	AM293054	Kauserud et al. 2007
BamEbw-109	Germany	No data	AM293059*	Kauserud et al. 2007
Isolate 81	New Zealand	<i>Podocarpus spicatus</i>	AM293060*	Kauserud et al. 2007
CCBAS 524	Czech Republic	No data	AM293061*	Kauserud et al. 2007
DAOM52883	India	<i>Pinus excelsa</i>	AM293064*	Kauserud et al. 2007
P167	Germany	Timber	AM946631*	Schmidt and Moreth 2008
Olrin 238	Lithuania	<i>Fraxinus excelsior</i>	AY787668*	Lygis et al. 2005
BI 516 (under <i>Coniophora</i> sp.)	Unknown	No data	EU162050*	Naumann et al. (unpublished)
LMSA1.03.047	Unknown	No data	GU066829	Maurice et al. (unpublished)
Isolate 8 (under <i>C. cerebella</i> = <i>C. puteana</i>)	USA	Conifer	GU187513	Binder et al. 2010
CFMR Isolate MAD-515	USA, Wisconsin	<i>Quercus</i> plank	GU187520†	Binder et al. 2010
MUCL 1000	Germany	No data	GU187521	Binder et al. 2010
BAM Ebw.15	Unknown	No data	EF524034*	Hogger et al. (unpublished)
C1RZ3	Italy	<i>Corylus avellana</i>	EU722763*	Pilotti et al. (unpublished)
MA-Fungi 60730, 15852Tell.	France	<i>Quercus pubescens</i>	HF921476	This study
Coniophora sp.				
Isolate BCP5436, PDD94245 (under <i>Coniophora</i> sp.)	New Zealand	No data	JF714651*	Johnston and Dickie (unpublished)
OUTGROUP				
Serpula himantioides (Fr.) P. Karst.				
P 99 (M213)	USA	No data	AJ245949*	Schmidt and Moreth 2000
Serpula lacrymans (Wulfen) J. Schröt.				
S7 (BAM 133)	Germany	No data	AJ245948*	Schmidt and Moreth 2000

(GenBank ITS nrDNA HG326617) • São Filipe, Caldeira do Fogo, Bangaeira; 14°58'40"N, 024°22'03"W; 1666 m alt., 22.IX.2010; M.T. Telleria leg.; on *Vitis vinifera*; 19015Tell.; MA-Fungi 98797 • São Filipe, Galinheiros; 15°00'08"N, 024°26'04"W, 319 m alt.; 24.IX.2010; M. Dueñas leg.; on *Saccharum officinarum*; 13284MD; MA-Fungi 86372 (GenBank ITS nrDNA HG326618).

Other material examined. USA – Arizona • Pinal

County, Santa Catalina Mountains, Canyon del Oro; R.L. Gilbertson leg.; RLG 10925; US 0290421, holotype, BPI! (GenBank ITS nrDNA HF921465).

Identification. Basidioma resupinate, widely effused and easily separated; hymenophore smooth, yellowish to olive brown (87. m. Y – 95. m. Ol Br); subiculum thin, white, cottony; margin cream to yellowish, arachnoid, with hyphal strands. Hyphal system monomitic,

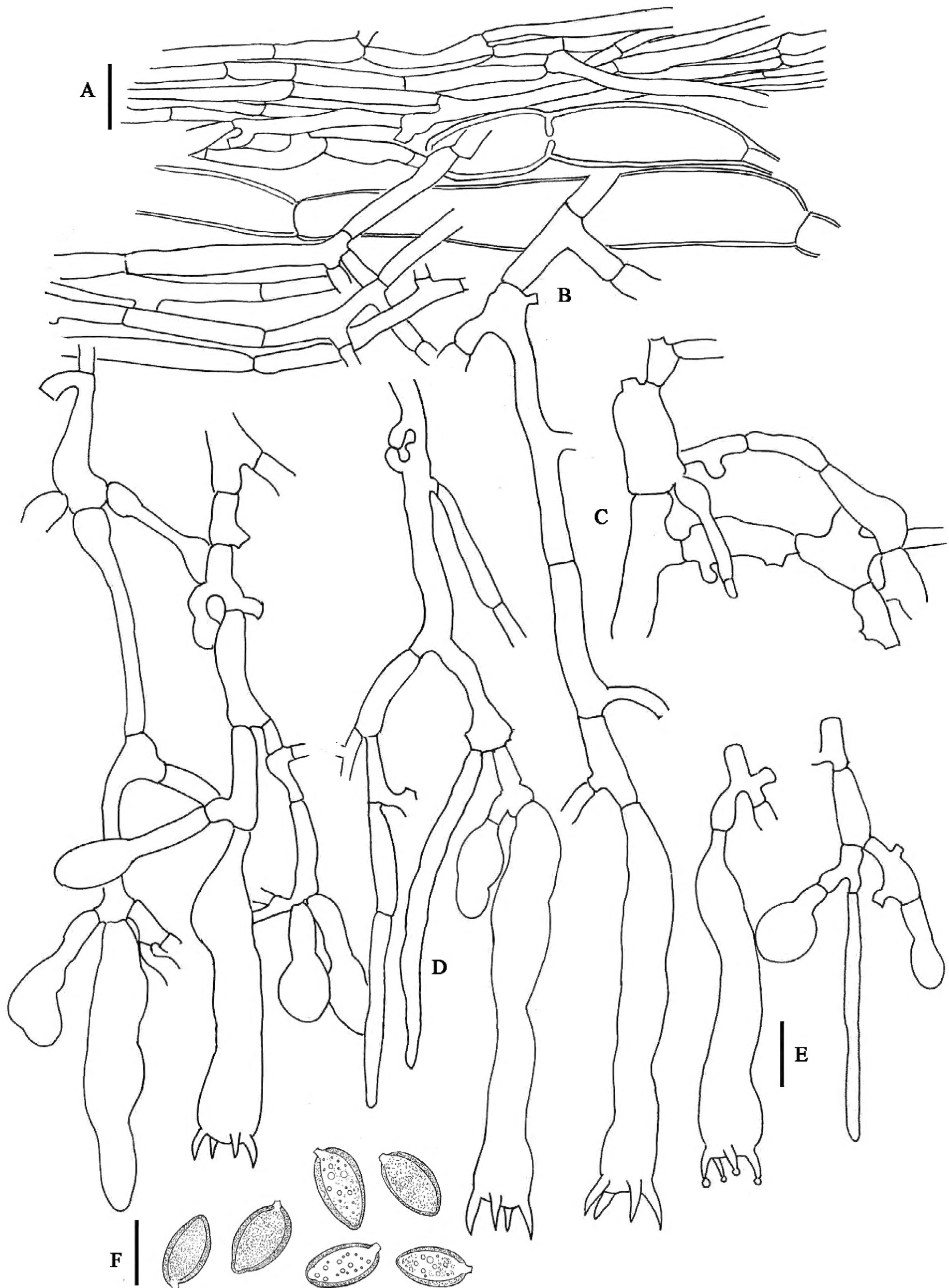


Figure 4. *Coniophora eremophila*, 13284MD, MA-Fungi 86372. **A.** Hyphal strands. **B.** Basal hyphae. **C.** Subhymenial hyphae. **D.** Hyphidia. **E.** Basidia. **F.** Basidiospores. Scale bar = 10 μm . (Drawing by M. Dueñas)

generative hyphae without clamps, clamps rare and only present on strands; strand hyphae thin to thick-walled, up to 14 μm wide; basal hyphae loosely interwoven, thin-walled, hyaline, 4–7 μm wide; subhymenial

hyphae more densely interwoven, short-celled, 8–13 μm wide. Cystidia absent. Hyphidia present, hyaline, up to 3 μm wide. Basidia cylindrical, sometimes pedunculate and sinuous, without basal clamp,

(45–)50–70(–100) × 6–7 μm, projecting up to 40 μm, four sterigmata up to 5 μm long. Spores ellipsoid, with prominent apiculus, 10–12 × 6–8 μm (Q = 1.6), thick-walled, yellowish, and dextrinoid.

The ITS dataset includes 80 sequences of *Coniophora* species, of which 19 were generated in this study. The two sequences obtained from Cape Verde specimens grouped together (Fig. 5), in a well-supported group (MP bs = 87; MLbs = 87%), with the five sequences from Arizona, including the sequence of the *C. eremophila* type, obtained in this work. Both morphological and

molecular analyses confirm that the specimens from Cape Verde belong to *C. eremophila*.

Discussion

Most of the species of *Coniophora* cause brown rot in all kinds of wood, producing economic losses in several ways: e.g. *Coniophora puteana* (Schumach.) P. Karst. is an important destroyer of building timber. Matheron et al. (1992) reported *Coniophora* species associated with brown heartwood rot in branches and trunks on lemon

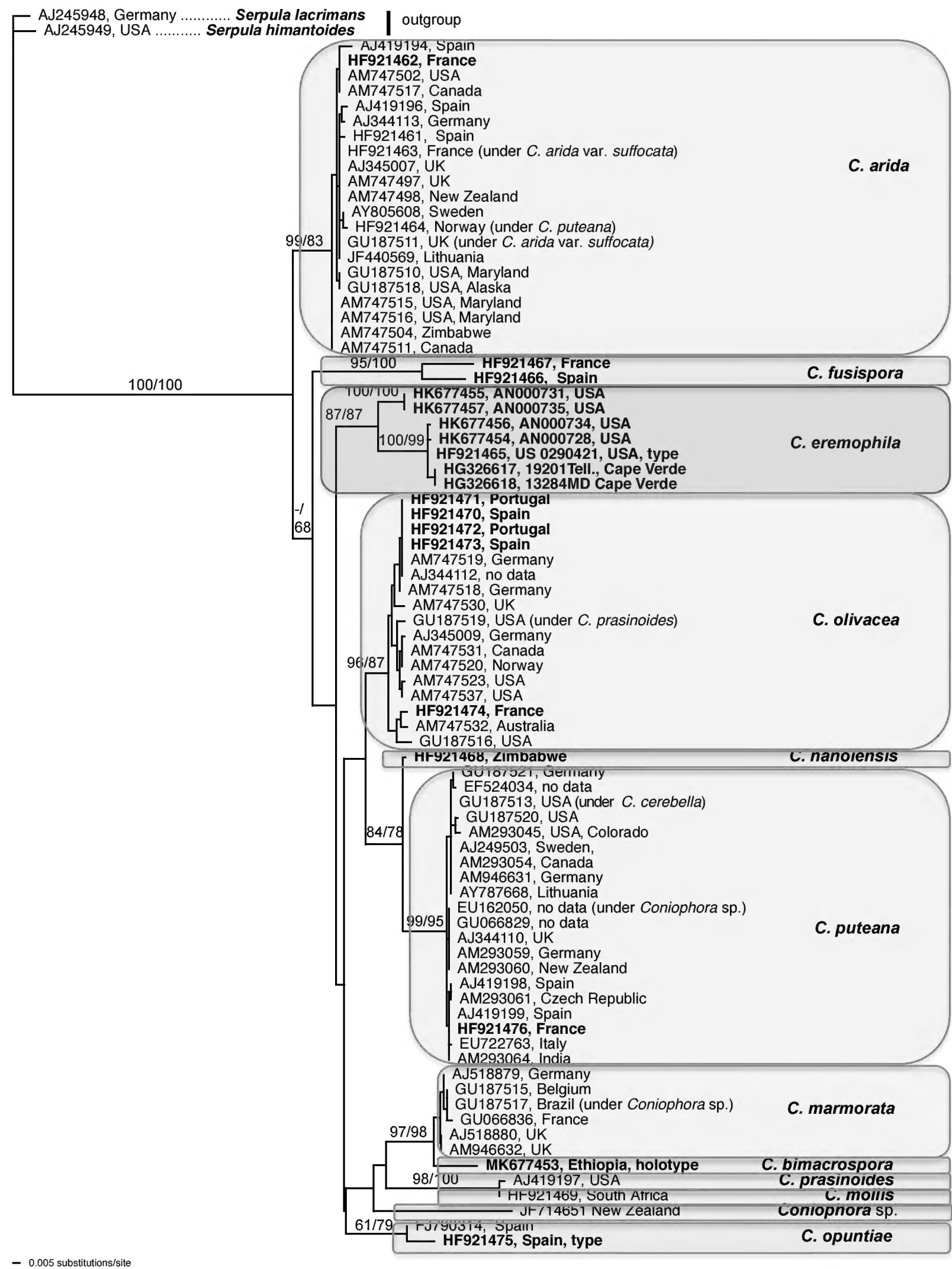


Figure 5. Phylogenetic tree obtained from the maximum-likelihood analysis of *Coniophora* species. Numbers above branches are parsimony (MPbs >50%) and maximum likelihood (MLbs >50%) bootstrap values. Each sequence with the accessions numbers from GenBank, and country. Newly generated sequences in bold. Clades named according to Index Fungorum and Mycobank.

trees in Arizona, USA, that was identified a few years later as *C. eremophila* (Bigelow et al. 1996). This record was the first of a species of *Coniophora* causing serious lesions in living citrus and other fruit trees. Several studies have been carried out on the biology, control, development, characterization, and molecular biology of this disease (Bigelow et al. 1996, 1998; Gilbertson et al. 1996; Wilcox et al. 1996; Demetriou et al. 2000; Adaskaveg et al. 2001; Matheron et al. 2006).

According to Mauk and Adaskaveg (2000), *C. eremophila* may be an opportunistic species disposed to colonize fruit trees weakened by other possible causes; our report offers additional information on this hypothesis, because it is also able to colonize other species of cultivated plants such as vines and sugarcane.

This study is first report of *C. eremophila* from the Cape Verde archipelago (Fig. 1) and extends the geographic distribution of this species by approximately 8,800 km in a straight line west to east from its previously known North American locality (Lindsey and Gilbertson 1975; Gilbertson et al. 1976) and 8,700 km southwest to northeast from the locality in Chile (Ginns 1982).

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Author Contributions

Conceptualization: MD, MPM, MTT. Data curation: MTT, MD. Formal analysis: MD, MPM, MTT. Funding acquisition: MTT. Investigation: MD, MPM, MTT. Methodology: MPM, MD. Project administration: MTT. Writing – original draft: MTT. Writing – review and editing: MD, MPM, MTT.

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